ORGANIC PROCESS RESEARCH & DEVELOPMENT
OPR&D
Article
Cite This: Org. Process Res. Dev. XXXX, XXX, XXX−XXX
pubs.acs.org/OPRD

Development of an Efficient Manufacturing Process to GSK2248761A API

Celine Bret,[†,§] Elaine Smith,[†] Erica Vit,[†,§] Jerome F. Hayes,[*,†] John D. Hayler,[†] Alan Ironmonger,[†] David Pascoe,[†] Neil Hodnett,[†] Jonathan Stanway,[†] Stephen Etridge,[†] Alistair Stewart,[‡,⊥] Jingyang Wang,[‡,⊥] Benjamin A. Mayes,[‡,⊥] and Adel Moussa[‡,⊥]

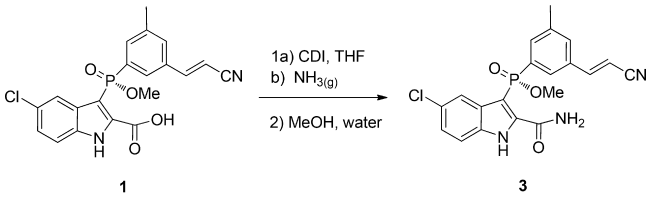
[†]GlaxoSmithKline, Medicines Research Center, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, U.K.

[‡]Merck & Co. Inc., 126 East Lincoln Avenue, Rahway, New Jersey 07065, United States

ABSTRACT: Amidation of indole 2-carboxylate **1** with ammonia gas via the imidazolide **2** gave GSK2248761A API **3**, which was in development for the treatment of HIV. Three significant impurities, namely the phosphinic acid **4**, the *N*-acyl urea **8**, and the indoloyl carboxamide **6**, were formed during the reaction, and the original process was unable to produce API within clinical specification when run at scale. Investigation into the origin, fate, and control of these impurities led to a new process which was able to produce API within clinical specification.

GSK2248761A (IDX899, Fosdevirine, **3**) is a potent non-nucleoside reverse transcriptase inhibitor that was in development as a potential treatment for HIV.[1] It was discovered by Idenix Pharmaceuticals, and a synthesis is described in the preceding paper. It used an unusual palladium catalyzed phosphorus−carbon coupling reaction to generate the entire molecular backbone. The final step of the synthesis involved the initial formation of an imidazolide intermediate **2** using CDI, followed by an aminolysis on addition of ammonia gas to the solution and was ostensibly straightforward (Scheme 1).[2]

Scheme 1. Preparation of GSK2248761A API from the Indole Acid 1



However, three significant impurities were formed during the reaction, the phosphinic acid **4**, the *N*-acyl urea **8**, and the indoloyl carboxamide **6** and the original process was unable to produce API within clinical specification when run on an 8 kg scale (Figure 1). In order to meet the challenging timelines for supply of API for clinical studies, it was necessary to scale-up the original process with minimal opportunity for process development. An understanding of the origin, control, and fate of these impurities was critical to enable us to accomplish this.

Origin and Control of Impurities. It was found that the phosphinic acid **4** was formed by overexposure of the product GSK2248761A to ammonia once the required reaction was complete. This was not of major concern, as **4** formed slowly on the reaction time scale and was readily removed by extraction with a pH 10 aqueous buffer. During the activation of the indole acid **1** with CDI, variable amounts of the diketopiperazide **5** were formed (Scheme 2). This intermediate was prepared independently and was shown to react with ammonia to give the indoloyl carboxamide **6**. Presumably this occurs via ring opening followed by acyl migration. Impurity **6** was found subsequently to react slowly with further ammonia to give GSK2248761A (Scheme 2).

Impurity **8** was not detected when the aminolysis was performed on a small scale (2 g). In contrast, the first pilot plant batch gave API containing 0.30% a/a of impurity **8** by HPLC, while the second batch gave 0.22% a/a impurity **8** by HPLC (Table 1, entries 1 and 2). It was strongly suspected that the ammonia gas supply rate was influencing impurity **8** formation. Traces of ammonia could promote N-acylation of indole with CDI. The resultant adduct **9** would then react with excess ammonia to form the hydantoin intermediate **7** which would then react with further ammonia to give **8** (Scheme 3). Alternatively ammonia might react with CDI to form carboxamide **10** which could react with **2**[3] to give the hydantoin **7**.

Addition of the required molar equivalents of ammonia took approximately 100 min in the pilot plant. The ammonia supply rate was limited by the constraints of the gas supply configuration in the plant. In a process stretching experiment, ammonia gas was added to a solution of the imidazolide **2** in THF and was then removed after a few seconds. Very high levels of impurity **8** (35% a/a by HPLC) were formed. In order to quantify the effect of ammonia gas supply rate on impurity **8** formation, two experiments were performed in an RC1 reactor on a 15 g scale (Figure 2). Ammonia was supplied via a mass flow controller and the supply rate was monitored using a mass flow meter, while the vessel occupancy was maintained at the same level as the pilot plant reactor. At an ammonia flow rate of 30 mL/min the solution level of impurity **8** was 0.54% a/a, and at 60 mL/min, 0.35% a/a **8** was obtained.

Received: November 10, 2017

DOI: 10.1021/acs.oprd.7b00357
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

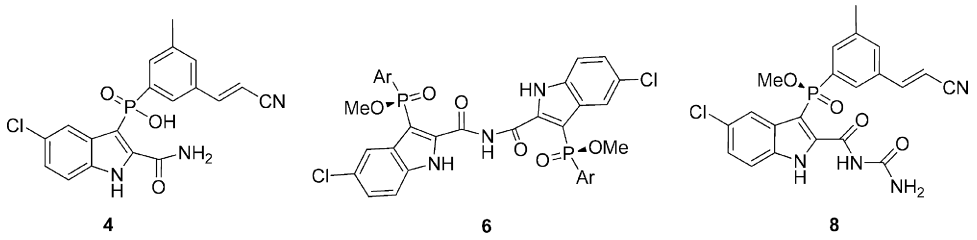


Figure 1. Structures of the main impurities.

Scheme 2. Formation of Indoloyl Carboxamide 6 and Its Conversion to GSK2248761A

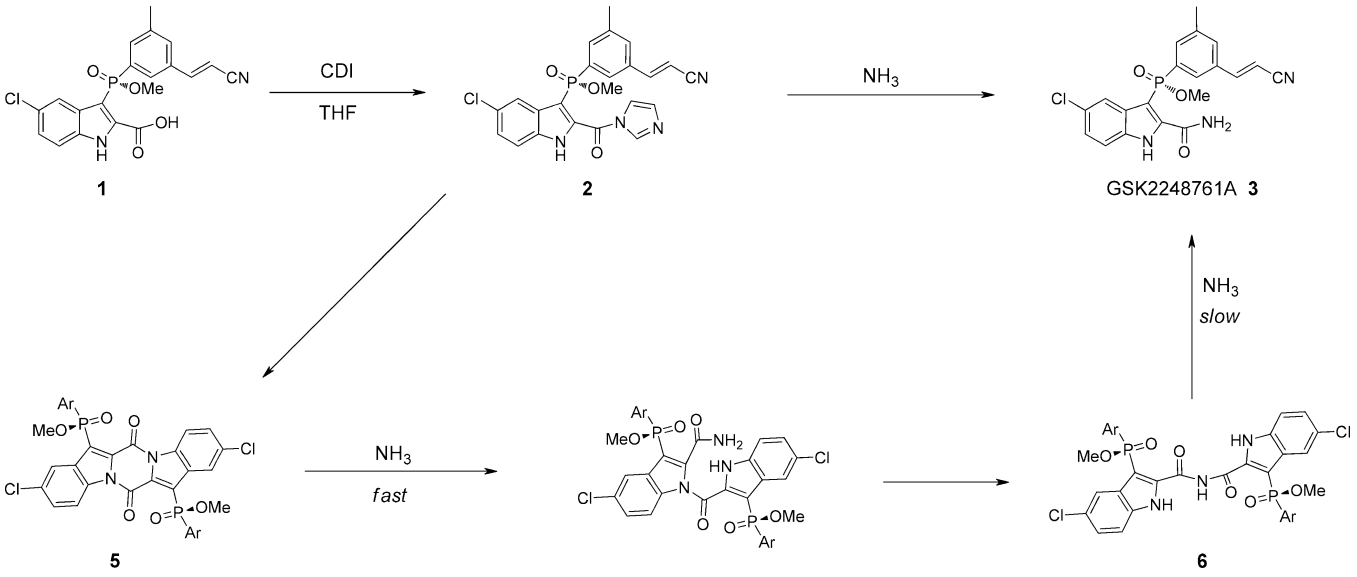


Table 1. Pilot Plant Data for the Conversion of Indole Acid 1 to GSK2248761A via Aminolysis of Imidazolide 2

Batch	1[a]	2[a]	3[b]	4[b]	5[b]	6[b]
scale (kg)[c]	8.1	21.0	20.0	26.1	17.2	17.5
yield (%)	82.2	83.7	89.0	87.5	87.1	97.8
purity (% area)[d]	99.24	99.67	99.83	100	99.88	99.88
4 (% area)	0.10	<0.05	ND	ND	ND	<0.05
8 (% area)	0.30	0.22	ND	ND	ND	ND
1 (% area)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
6 (% area)	<0.05	ND	<0.05	<0.05	<0.05	<0.05

[a]Addition of ammonia to the imidazolide. [b]Addition of the imidazolide to ammonia. [c]Scale wrt indole acid **1**. [d]See ref 4 for HPLC conditions for API impurity profile.

The 30 mL/min supply rate was a direct scale down of the ammonia gas supply rate achieved on plant scale where the solution level of impurity **8** was 0.54% a/a. The results obtained strongly support the correlation between the level of impurity **8** formed in the reaction and ammonia supply rate. It was evident therefore that the ammonia supply rate would need to be increased significantly to deliver API within clinical specification (0.15% a/a **8**) using the original process.

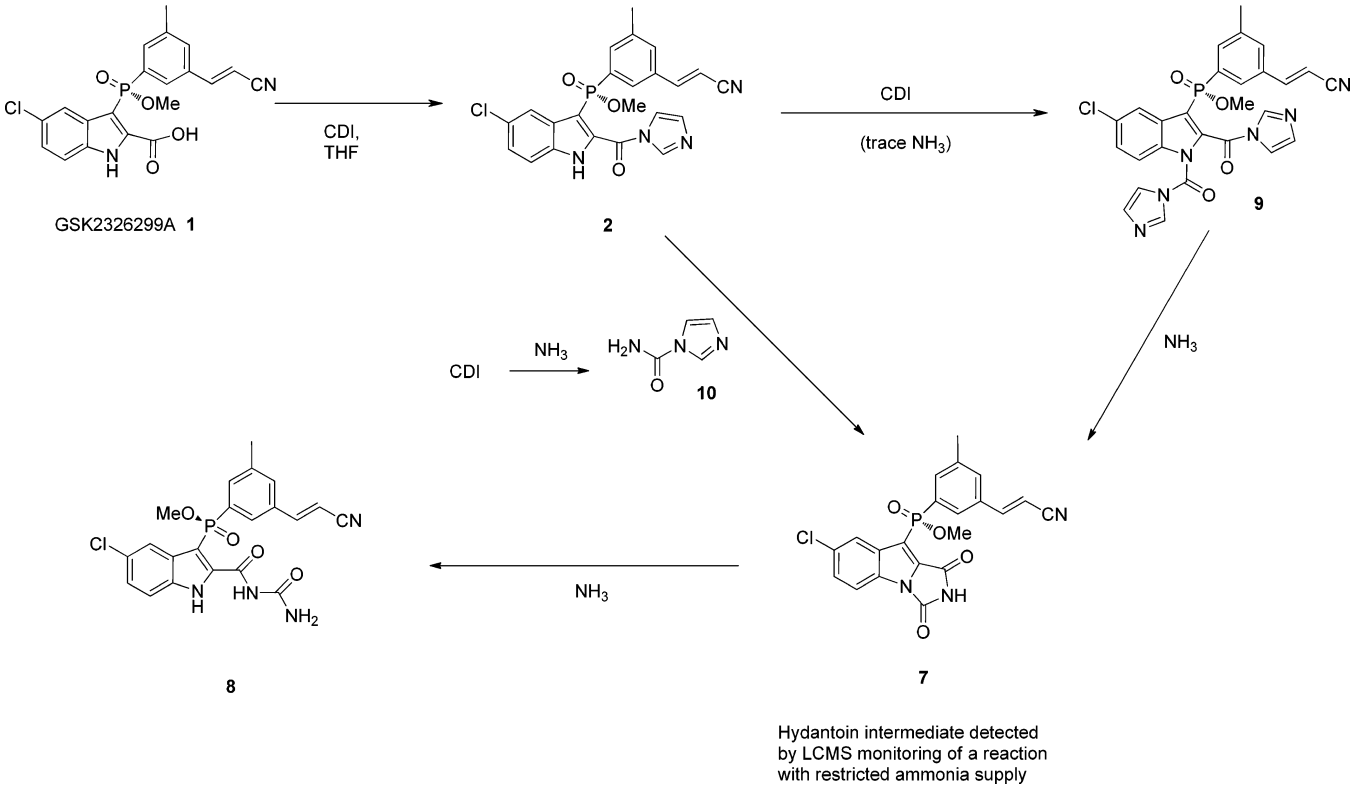
To circumvent this engineering challenge, we considered inverse addition, i.e. addition of the imidazolide solution to an ammonia gas/THF mixture. This would ensure that the imidazolide was always exposed to a large excess of ammonia. Several inverse addition experiments were run in a 1 L reactor maintaining the ammonia pressure between 0.2 and 0.4 barg, and as predicted no impurity **8** was formed provided the equipment was configured to prevent ingress of ammonia gas into the bulk imidazolide feed solution. The inverse addition process was then performed on a pilot plant scale (20 kg), and consistently no impurity **8** was formed (Table 1 batches 3–6). However, to enable safe operation within the plant vessel pressure limits while avoiding the risk of starving the reaction of ammonia, several interruptions to the imidazolide addition were required in order to repressurize the vessel with ammonia. For later batches this issue was circumvented by evacuating the vessel headspace prior to charging ammonia. Given the variation in operating limits between individual vessels and production facilities, it was felt that this process was not amenable to straightforward changes in scale and equipment and therefore an improved process was sought.

Process Optimization. Having successfully supplied the API required for clinical studies we were able to focus on improvements to this process with respect to long-term manufacturability. Of specific concern was the use of ammonia gas and the overall volume inefficiency of the process (maximum 90 volumes).

Addition of a solution of imidazolide **2** to a mixture of dilute aqueous ammonia (less than 28% v/v) (8 equiv) in THF gave an increased amount of hydrolysis (i.e., unreacted indole acid **1**) and an increased amount of indoloyl carboxamide **6**. However, using 28% aqueous ammonia (8 equiv) we were able to isolate GSK2248761A within specification. In addition, raising the temperature to 40–50 °C gave an increased rate of conversion of intermediate **6** to **3**, whereas with ammonia gas in

DOI: 10.1021/acs.oprd.7b00357
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Scheme 3. Proposed Mechanism for the Formation of the *N*-Acyl Urea Impurity 8



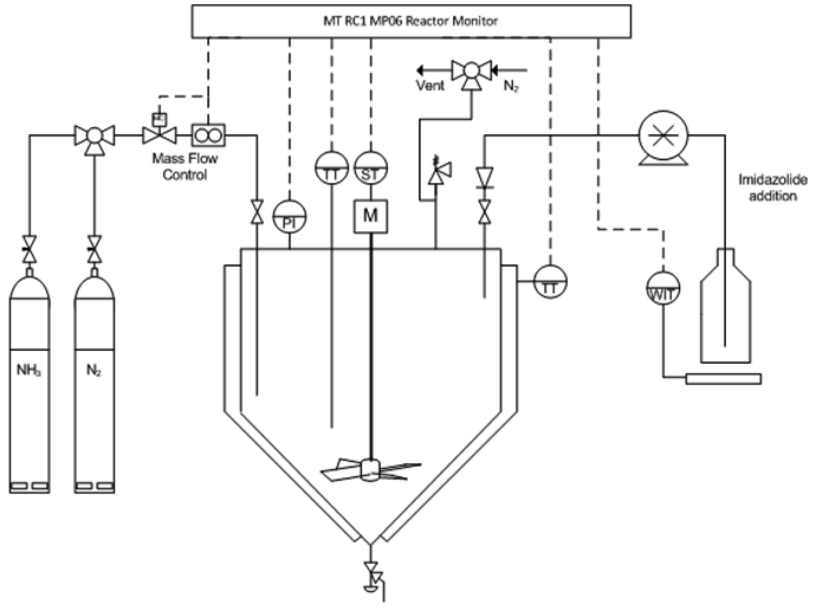


Figure 2. Experimental configuration used to study the aminolysis reaction. Nitrogen was used to purge and inert the vessel. For the inverse addition experiments a nonreturn valve was installed on the imidazolide feed line to prevent ingress of gaseous ammonia. Process sensors are shown as labeled circles (PI = pressure indicator, TT = temperature transmitter, ST = stirrer speed transmitter, WIT = weight indicator and transmitter).

THF, an increase in temperature led to an increase in formation of impurity **8**. This could be rationalized on the basis of decreased solubility of ammonia at elevated temperatures under anhydrous conditions. Substituting THF with 2-methyltetrahydrofuran also significantly improved the process. Due to the immiscibility of 2-methyltetrahydrofuran with water, it was no longer necessary to use TBME in the aqueous workup. Furthermore, it was possible to increase the reaction concentration from 40 volumes to 15 volumes with no adverse effect on product purity. At this higher concentration, imidazolide **2** dimerized to the diketopiperazide **5** in greater amounts than was previously observed. Subsequent reaction monitoring demonstrated that under these conditions diketopiperazide **5** reacted efficiently with concentrated aqueous ammonia to give GSK2248761A within specification. These observations enabled us to design a significantly more

DOI: 10.1021/acs.oprd.7b00357
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

volume efficient process and avoided the use of gaseous ammonia entirely.

CONCLUSION

A pilot plant process which was capable of producing the potent non-nucleoside reverse transcriptase inhibitor GSK2248761A within clinical specification was successfully developed. This was subsequently optimized on a laboratory scale with an intricate knowledge of impurity origin to give a significantly more volume efficient process which avoided the operational challenges associated with gaseous ammonia.

EXPERIMENTAL SECTION

General Experimental. Reactions were monitored using HPLC on a Luna C18(2), 50 mm × 2 mm, 3 μm, eluting with gradient 0% to 95% (0.05%v/v TFA in water to 0.05%v/v TFA in acetonitrile) over 8 min at 40 °C detecting at 220 nm. NMR spectra were recorded on a Bruker 400 mHz Ultrashield, or a Bruker AV500 spectrometer. HRMS data were recorded on an LQT Orbitrap spectrometer. Infrared spectra were recorded on a PerkinElmer Spectrum One spectrometer.

Pilot Plant Preparation of GSK2248761A 1 (Batch 5). To a solution of *N,N*-carbonyldiimidazole (13.34 kg, 82.27 mol) in THF (229 kg) was added GSK2326299A (17.2 kg, 41.5 mol) as a solution in THF (153 kg) at 19−25 °C over 30 min. Further THF (38 kg) was then added. The resultant mixture was warmed to 38 °C over 30 min and was maintained at this temperature for 2 h. The resultant clear yellow solution was then cooled to 5−15 °C over 1 h. The reactor was then evacuated 4 times and purged with nitrogen to remove carbon dioxide.

A second reactor was charged with THF (153 kg), and the solvent was cooled to 0−10 °C. The reactor was then evacuated to −0.85 barg and then was charged with ammonia gas (4.9 kg, 287.7 mol) over 100 min. The imidazolide solution in THF was then transferred to the reactor containing ammonia in THF over 30 min at 0−10 °C. A differential pressure of 0.5 barg was maintained between the two reactors to prevent ammonia transfer to the bulk imidazolide solution. A 38 kg line wash of THF was then transferred from the first reactor to the aminolysis reactor. The reaction mixture was then warmed to 17−27 °C over 1 h. The reaction mixture was stirred at this temperature for 2.5 h after which time further ammonia (0.7 kg, 41.1 mol) was added. The reaction mixture was stirred at 17−27 °C for a further 17 h after which time the level of imide **6** fell below the target of 0.2a/a %. The reactor was then purged with nitrogen, and the reaction mixture was cooled to 10 °C. Hydrochloric acid (171 kg of a 2 M aqueous solution) was then added at 10−15 °C over 80 min. *tert*-Butylmethyl ether (508 kg) was then added, and the mixture was stirred at 15−25 °C for 25 min. The organic layer was separated and was washed with water (172 kg), aqueous pH 10 buffer solution (172 kg containing 0.96 kg of sodium hydroxide and 4.7 kg of sodium bicarbonate in 166 kg water), and water (172 kg). The organic layer was then concentrated by vacuum distillation to a total weight of 72 kg. Methanol (97 kg) was then added, and a further 103 L of solvent were removed by vacuum distillation. The put and take distillation was repeated with two further 81 kg batches of methanol. Water (13 kg) was then added over 30 min, and the mixture was stirred at 15−25 °C for 1 h. The resultant slurry was filtered and washed with methanol/water 3:2 (51 kg). The product was dried at 50 °C under vacuum in a 0.6 m^2 agitated filter drier and was obtained as a white crystalline solid. Yield = 15.6 kg, 87%. HPLC (99.88%);[4] ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 2.37 (s, 3H), 3.86 (d, J = 15.0 Hz, 3H), 5.86 (d, J = 15.0 Hz, 1H), 5.94 (s, 1H), 7.33 (dd, J = 9.0 Hz, J = 2.0 Hz, 1H), 7.34 (d, J = 15.5 Hz, 1H), 7.39 (s, 1H), 7.49 (dd, J = 9.0 Hz, J = 1.5 Hz, 1H) 7.60 (d, J = 13.5 Hz, 1H), 7.64 (d, J = 13.5 Hz, 1H), 7.65 (d, J = 1.5 Hz, 1H), 10.40 (s, 1H), 10.88 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 21.3, 52.1, 98.1, 100.5 (d, J = 152.5 Hz), 113.9, 117.6, 120.9, 126.2, 126.5 (d, J = 11.3 Hz) 128.7, 129.9 (d, J = 10.1 Hz), 131.7, 133.0 (d, J = 151.2 Hz), 133.2 (d, J = 8.8 Hz), 133.4 (d, J = 10.1 Hz), 134.1 (d, J = 15.1 Hz), 138.7, 139.9, 149.2 and 161.2; ^{31}P NMR (202 MHz, $CDCl_3$) δ 31.4.

IR ν (cm^{-1}) 3280, 3065, 1679, 1619, 1402, 1195 and 1010.

HRMS calcd for $C_{20}H_{18}ClN_3O_3P$: 414.0769; HRMS found $[M + H]^+$: 414.0760.

Improved Process to GSK2248761A. To a stirred suspension of *N,N*-carbonyldiimidazole (7.8 g, 48.2 mmol) in 2-methyltetrahydrofuran (150 mL) was added GSK2326299A (10.0 g, 24.1 mmol) over 5−10 min at 25 °C. The temperature was adjusted to 34−37 °C, and the solution was stirred for 2 h. The resultant yellow solution was then cooled to 25 °C, and it was added to a separate, pressure-rated vessel containing a 28−30% aqueous solution of ammonium hydroxide (12.9 mL, 193.3 mmol) at 3−10 °C over 15 min. The resultant mixture was warmed to 25 °C over 15 min and was then warmed to 40−42 °C. The mixture was stirred for 2 h after which time the solution was cooled to 5 °C and water (40 mL) was added. Concentrated hydrochloric acid (40 mL) was added slowly at 5−20 °C to adjust to pH 1.0−1.5. The organic phase was separated and was washed with brine (50 mL of a 1% w/w aqueous solution). The organic phase was then washed with a pH 10 sodium bicarbonate buffer containing 1% w/w sodium chloride (2 × 50 mL). The organic phase was diluted with 2-methyltetrahydrofuran (60 mL), and solvent (120 mL) was distilled off. The resultant azeotropically dried solution was allowed to cool to room temperature and was filtered. Methanol (60 mL) was added, and solvent (60 mL) was distilled off. This was repeated twice to give a final volume of 60 mL. The solution was cooled to rt after which point the product crystallized. Water (26 mL) was then added, and the resultant slurry was stirred for 30 min. The product was filtered, washed with MeOH/water 2:1, and dried under a stream of nitrogen at room temperature. Yield = 8.0 g (81%), HPLC 99.85%.

Preparation of Phosphinic Acid 4. To a solution of GSK2248761A **1** (0.66 g, 1.59 mmol) in methyl isobutyl ketone (25 mL) was added tetrabutylammonium bromide (2.0 g, 6.2 mmol). The reaction mixture was heated to 100 °C to give a colorless solution. After 3 h, further tetrabutylammonium bromide (2.0 g, 6.2 mmol) was added, and the reaction was continued for a further 2 h. The reaction mixture was then cooled to rt and was washed with sulfuric acid (50 mL of a 1 M aqueous solution and 100 mL of a 1 M aqueous solution). The organic layer was then extracted with sodium bicarbonate (2 × 50 mL of a 6% w/w aqueous solution). The combined aqueous extracts were acidified to pH 1.0 to give phosphinic acid **4** as a white precipitate. This was filtered off and was recrystallized from methanol (5.0 mL). Yield = 0.15 g, 24%. ^{1}H NMR (500 MHz, DMSO) δ 2.23 (s, 3H), 6.38 (d, J = 16.5 Hz, 1H), 7.18 (dd, J = 8.7 Hz, J = 2.5 Hz, 1H), 7.43 (m, 1H), 7.46 (dd, (br), 1H), 7.58 (s, 1H), 7.61 (d, 1H), 7.66 (d, 1H), 7.69 (d, J = 1.8 Hz, 1H), 7.79 (s (br), 1H), 10.49 (s (br), 1H) and 12.51 (d, J =

DOI: 10.1021/acs.oprd.7b00357
Org. Process Res. Dev. XXXX, XXX, XXX−XXX

2.4 Hz, 1H). IR ν (cm^{-1}) 3440, 2920, 1668, 1620, 1402, 1054, and 1028.

HRMS calcd for $C_{19}H_{16}ClN_3O_3P$: 400.0612; HRMS found $[M + H]^+$: 400.0608

Preparation of *N*-Acyl Urea 8. To a solution of GSK2248761A (0.57 g, 1.38 mmol) in acetonitrile (7.5 mL) and THF (7.5 mL) was added *N*-chlorosulfonyl isocyanate (0.12 mL, 1.38 mmol). The reaction mixture was stirred at rt for ca. 3 h and was then quenched with water (15 mL). Organic solvent was then removed *in vacuo*, and the aqueous mixture was extracted with ethyl acetate (2 × 75 mL). The organic extract was concentrated to dryness *in vacuo*, and the residue was chromatographed on silica gel using ethyl acetate/heptane 4:1 as eluant. The required product was obtained as a white solid (0.12 g, 42%). ^{1}H NMR (500 MHz, DMSO) δ 2.34 (s, 3H), 3.79 (d, 3H), 6.52 (d, 1H), 7.36 (dd, 1H), 7.55 (d, 1H), 7.56 (s br, 1H), 7.64 (dd, 1H), 7.66 (d, 1H), 7.67 (d, 1H), 7.74 (m, 1H), 7.84 (d, 1H), 7.94 (s br, 1H), 12.75 (s, 1H), and 13.05 (s, 1H).

IR ν (cm^{-1}) 3401, 3207, 2944, 2860, 1714, 1660, 1588, 1400, and 1231.

HRMS calcd for $C_{21}H_{19}ClN_4O_4P$: 457.0827; HRMS found $[M + H]^+$: 457.0821.

Preparation of Indoloyl Carboxamide 6. To a solution of diketopiperazide **5** (500 mg, 0.630 mmol) in 2-methyltetrahydrofuran (2-MeTHF (7.5 mL) was added ammonium hydroxide (0.8 mL of a 25% w/w aqueous solution, 5.0 mmol). The resultant mixture was stirred at room temperature until the bright yellow color had disappeared (ca. 30 min). After this time the reaction mixture was added to a 1:1 mixture of water/2-methyltetrahydrofuran (100 mL). The organic layer was separated and was concentrated to dryness to give a 7:3 mixture of **6**/GSK2248761A (455 mg, 63%). An analytically pure sample was prepared using mass directed preparative chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 6H), 3.86 (d, 6H), 5.88 (d, 2H), 7.26 (d, 2H), 7.36 (m, 4H), 7.60 (d, 2H), 7.65 (d, 2H), 7.80 (d, 2H), 7.89 (s, 2H), 11.56 (s, 2H), and 13.90 (s, 1H).

IR ν (cm^{-1}) 3239, 1730, 1543, 1403, 1317, 1202, and 1025.

HRMS calcd for $C_{40}H_{32}Cl_2N_5O_6P_2$: 810.1199; HRMS found $[M + H]^+$: 810.1197.

Preparation of Diketopiperazide 5. To a suspension of CDI (7.82 g, 48.23 mmol) in 2-Me-THF (100 mL) was added **1** (10 g, 24 mmol) over 5 min at rt. The resultant mixture was warmed to 39 °C and was stirred for 2 h. The reaction mixture was then allowed to cool to rt, and the resultant suspension was stirred for 20 h. The product was filtered and washed with 2-MeTHF (ca. 20 mL) and was dried at 25 °C for 3 h. The product was obtained as a bright yellow solid (7.89g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (s, 6H), 3.89 (d, 6H), 5.96 (d, 2H), 7.38 (d, 2H), 7.40 (s, 2H), 7.62 (d, 2H), 7.76 (d, 2H), 7.93 (d, 2H), 8.52 (d, 2H), and 8.64 (s, 2H); IR ν (cm^{-1}) 1709, 1540, 1447, 1359. 1167, 1120, 1066, 1029, and 965.

HRMS calcd for $C_{40}H_{29}Cl_2N_4O_6P_2$: 793.0934; HRMS found $[M + H]^+$: 793.0926

AUTHOR INFORMATION

Corresponding Author

*E-mail: Jerome.Hayes@gsk.com.

ORCID

Jerome F. Hayes: 0000-0001-9875-0464

John D. Hayler: 0000-0003-3685-3139

Notes

The authors declare no competing financial interest.

[§]C.B. and E.V. are former GlaxoSmithKline employees.

[⊥]A.S., J.W., B.A.M., and A.M. are former Idenix employees.

REFERENCES

(1) (a) Klibanov, O. M.; Kaczor, R. *Current opinion in investigational drugs (Biomed central)* **2010**, *11* (2), 237−245. (b) Zhou, X.-J.; Pietropaolo, K.; Damphousse, D.; Belanger, B.; Chen, J.; Sullivan-Bolyai, J.; Mayers, D. *Antimicrob. Agents Chemother.* **2009**, *53*, 1739. (c) Storer, R.; Dousson, C.; Alexandre, F.-R.; Roland, A. US 2006 0074054 A1. (d) Castellino, S.; Groseclose, M. R.; Sigafoos, J.; Wagner, D.; de Serres, M.; Polli, J. W.; Romach, E.; Myer, J.; Hamilton, B. *Chem. Res. Toxicol.* **2013**, *26*, 241.

(2) (a) Storer, R.; Alexandre, F. R.; Dousson, C.; Moussa, A. M.; Bridges, E. PCT International Application WO2008/042240A2, 2008. (b) Alexandre, F. R.; Amador, A.; Bonaric, S.; Bot, S.; Caillet, C.; Da Costa, D.; Lioure, M. P.; Mayes, B. A.; Parsy, C.; Roland, A.; Rosinovsky, E.; Stewart, A.; Wang, J.; Moussa, A.; Dousson, C. *236th ACS National Meeting in Philadelphia* 17−21 August 2008.

(3) Macor, J. E.; Cuff, A.; Cornelius, L. *Tetrahedron Lett.* **1999**, *40*, 2733.

(4) HPLC method: Kinetrix 2.6 μm C18 100A, 4.6 mm × 100 mm; flow: 2.4 mL/min; eluant A: water with 0.25% v/v TFA; eluant B: acetonitrile with 0.25% v/v TFA; Gradient: 0 min: 80% A, 20% B; 55 min: 47% A, 53% B; 55.1min: 80% A, 20% B; 60 min: 80% A, 20% B. UV detection at 275 nm.

DOI: 10.1021/acs.oprd.7b00357
Org. Process Res. Dev. XXXX, XXX, XXX−XXX